PB-228 228

A CRITICAL STUDY OF ANALYTICAL METHODS FOR SEA WATER

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Prepared for:

Public Health Service

31 May 1965

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PB 228 228

USPHS Grant No. WP 00339

A CRITICAL STUDY OF ANALYTICAL METHODS FOR SEA WATER

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Final Report

May 31, 1965

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Bronx, N.Y. 10453

Final Report

A. SUMMARY PAGE

Grant Number:	WP 00339
Title:	A Critical Study of Analytical Methods for Sea Water
Principal Investigator:	Dr. Gail P. Edwards
Institution:	New York University
Period Covered:	June 1, 1962 - May 31, 1965
Date:	May 31, 1965

Summary Statement

Work has been done toward developing analytical procedures for use in brackish and sea waters. These procedures will be of great help in studying pollution and growth of phytoplankton in tidal bays, harbors and rivers.

1. A nitrate test developed for water and wastewater appeared suitable for use in salt water.

2. Preliminary tests indicate that the fresh water kjeldahl procedure for organic nitrogen may not be satisfactory for use in salt waters.

3. An orthophosphate test was developed by modification of and addition to an existing reagent system. The test depends upon the formation of the intensely colored molybdenum blue using ascorbic acid as a reducing agent and an antimony salt to speed up the reaction and intensify the color. Alcohol is added to extend the range over which the reaction follows Beer's Law. This test has proved reliable and follows Beer's Law in distilled and sea water, sewage and effluents up to at least 2 mg/1 PO_4 reported as P.

4. Automated analytical equipment has been successfully used in the development of manual analytical methods.

B. DETAILED REPORT

Increased attention is being given to pollution studies in waters of various salinities ranging from brackish to sea water. The Standard Methods for Examination of Water and Wastewater[1] was developed for fresh water and there are no generally approved methods of analysis for many of the important constituents in brackish and sea waters. In the oceanographic field there is no set of standard procedures and laboratories may use different procedures for making a given determination.

It was the object of this work to establish accurate, precise analytical procedures for use in brackish and sea waters. These procedures will be of great help in studying pollution of tidal bays, harbors and rivers, and phytoplankton growth on beaches. The Joint Editorial Board for the twelfth edition of "Standard Methods" recognizes the need for acceptable methods for use in salt waters and plans to incorporate the available methods in the next edition.

The detection of phosphorus and nitrogen is of great importance in water pollution work because sewage and some industrial wastes supply these nutrients required for the objectionable excessive growth of algae and other phytoplankton in fresh and salt waters. As a result, the experimental work has been concentrated on nitrate nitrogen and orthophosphate phosphorus analysis.

NITRATE

The first phase of this project (WP-00339) was in adapting to sea water a nitrate test[2] developed in this laboratory for water and wastewater from work on U.S.P.H.S. Grant RG 4649. Standard Methods does not contain a suitable method for low concentrations of nitrate in salt water but

there were two procedures which seemed worthy of investigation for use in salt water. Both were dependent upon reduction of nitrate to nitrite, diazotization and comparison of color developed with standards.

The nitrate test[2] developed for water and wastewater seemed promising for saline waters since one of the reagents was zinc diluted with solid sodium chloride. Tests were made using distilled water samples containing standard nitrate solution and various concentrations of chloride. Calibration curves prepared from samples containing 5,000, 10,000 and 20,000 mg/l of chloride as Cl^- followed Beer's Law and were essentially the same as those prepared from fresh water samples. The added amount of chloride was in addition to that contained in the zinc reagent.

Preliminary tests were also made using the method of Strickland and Parsons[3] as published in their book "Manual of Sea Water Analysis". This method depends upon the reduction of nitrate to nitrite with hydrazine (catalyzed with copper) at pH 9.6, followed by diazotization and coupling with N(1-naphthyl)-ethylene diamine. The color formed is compared with standards. The method seemed satisfactory but is very time consuming since during the procedure, the sample must stand eight hours or more at one stage and twelve hours at another stage.

Organic Nitrogen

Organic nitrogen according to Standard Methods is determined by the kjeldahl method using mercuric sulfate as a catalyst. Nitrogen in the trinegative state is converted to ammonium bisulfate by digestion with sulfuric acid to which potassium sulfate has been added to raise the boiling point to 345-370°C. The temperature should not exceed 382°C. or loss of nitrogen results.

The salt/acid ratio is important in regulating the boiling point of the digesting liquor. The Standard Method recommends a ratio of 0.67 grams K_2SO_4 per ml of concentrated sulfuric acid. The recommended 50 ml of digestion solution contains 6.7 grams K_2SO_4 and 10 ml of concentrated H_2SO_4. If 500 ml sample of sea water is used in the determination, about 16 grams of salt, mainly chloride, would be added to the digestion mixture. Hydrochloric acid will be driven off and the proportion of salt to acid will increase significantly.

A few exploratory tests were made. Temperatures were measured with a miniature wire type thermocouple of iron and constantan connected to a temperature potentiometer. In one test, 0.4 grams of dehydrated nutrient broth was diluted to 500 ml with sea water and digested with 50 ml of digestion solution. The sample charred and the temperature rose to 500°C. In a sample run in the same way with distilled water, the temperature reached 381°C. The digestion flask in the first experiment contained two openings through which vapor escaped. The experiment with sea water was repeated using a digestion flask with only one opening to retard the loss of sulfuric acid fumes. The temperature rose to more than 500°C. and the tip of the stainless steel thermocouple corroded so much that it became useless.

These experiments are far from conclusive but they do indicate the danger in using a fresh water kjeldahl method for a sea water. Much more work will be needed to confirm the results of these experiments and to set up a satisfactory procedure for salt water samples.

ORTHOPHOSPHATE

The Standard Methods[1] describes two methods for the determination of orthophosphate which were developed for use in fresh waters. Both depend upon the formation of an

intensely colored molybdenum blue which results from the reduction of phosphomolybdic acid. The reducing agents are: amino naphthol sulfonic acid in one method and stannous chloride in the other. According to Standard Methods, the amino naphthol sulfonic acid method is useful in the range of 0.033 to 10 mg/l P but may give low results in highly saline waters. The stannous chloride method is suitable for the range 0.02 to 1 mg/l P. Burton and Riley[4] have pointed out that the stannous chloride reagent is not stable, the time for maximum color development varies with the temperature and salinity of the water, the color fades rapidly and there is a fairly large salt error.

Several methods have been proposed by the oceanographers for the determination of orthophosphate in sea water but no one method has been selected as a standard. Several oceanographic laboratories are using a method proposed by Murphy and Riley[5] which uses ascorbic acid as the reducing agent. Although the reduction of phosphomolybdic acid to molybdenum blue by ascorbic acid was reported by Ammon and Hinsberg[6] in 1936, it was first suggested for use in sea water in 1954 by Greenfield and Kalber.[7] Alteration of the method to provide a single reagent for the phosphate determination in sea water was described in 1958 by Murphy and Riley.[8] The reagent contained sulfuric acid, ammonium molybdate and ascorbic acid. The color which developed in 24 hours at room temperature, and in 30 minutes at 60°C., was stable for several days. They reported that Beer's Law was followed up to a concentration of at least 0.6 mg/l in sea water with a salt error of about four per cent.

Sea waters contain organic phosphorus compounds which may be hydrolyzed to orthophosphate during the long period or by the high temperature (60°C.) needed for color formation. To minimize the danger of hydrolysis, Murphy and Riley[5]

further revised the method by adding an antimony salt to the single reagent to reduce the time of color development to ten minutes at room temperature. The color was stable for at least 24 hours. They reported that Beer's Law was followed to at least 0.25 mg/l PO_4 as P and the salt error was less than one per cent. Although this concentration range reported by Murphy and Riley is satisfactory for ocean waters, it would not be satisfactory in waters near populated or agricultural areas where phosphate concentrations may exceed that concentration. Therefore, experimental studies were begun with the objectives of (a) extending the concentration range of orthophosphate beyond that stated by Murphy and Riley, and (b) intensifying the color formed to make the method more sensitive at low orthophosphate concentrations.

Experimental Method

During the phosphate study, many analyses were made with an automated wet chemical analyzer (an AutoAnalyzer) which by speeding up the work, permitted us to try many modifications and run replicates for determining precision. The use of the AutoAnalyzer was first made available by a short-term equipment rental grant from the Edanros Research Corporation. Later an AutoAnalyzer was purchased through U.S.P.H.S. Grant No. WP 00339-02S1.

Methods must be developed primarily for manual means since that is still the common procedure; therefore, the results were checked manually. It was found that in general, the results of automated analysis were transferable to manual testing although this was not always the case. This was primarily due to the fact that a precipitate was formed at room temperature at the higher phosphate concentrations which affected the automated method more severely than the manual method. This in no way detracts from the advantages of the

automated analytical equipment, i.e., speed and ability to handle a large number of samples, thus making it possible to do many replicate samples and to make variations in the procedure and testing the result without overloading the chemist. Then only the best of the variations needs to be checked manually. The automatic equipment for this project has permitted us to test methods and modifications rapidly so as to speed up the development of manual methods.

Manual Analysis of Water Samples

In these studies, unless otherwise stated, the measurements of absorbance were made with a Beckman Model B spectrophotometer at a wave length of 880 mμ using a one centimeter light path.

Our initial studies on the Murphy and Riley[5] method showed that Beer's Law was followed to a concentration of about 1.20 mg/l PO_4-P in both distilled water and sea water. The curves were nearly identical. In order to increase the depth of color produced so as to make the method more sensitive in low concentrations of phosphate and to extend the range beyond the 1.20 mg/l limit of Murphy and Riley, it was necessary to modify the reagents. It was found that the color was intensified by increasing the amount of antimony and decreasing the amounts of sulfuric acid, ammonium molybdate, and ascorbic acid added. With this new modified mixed reagent, the absorbances were generally increased 10-15%.

Work with the modified mixed reagent showed that the upper Beer's Law limit was still about 1.20 mg/l PO_4-P for distilled water and for sea water. In sea water at higher concentrations of phosphate, a precipitate was formed which interfered with the colorimetric determination. The precipitate seemed to be an antimony phosphorus molybdenum blue complex.

A reagent system was therefore sought which would eliminate the formation of the precipitate which formed at the higher phosphate levels, particularly in saline water. On the premise that the color precipitate was due to some form of molybdenum blue-antimony chloride, experiments were initiated with the aim of increasing the solubility of the antimony chloride complex.

It was found that a test temperature of 75°C. yielded good results. However, because of hydrolysis of condensed and organic phosphates at this elevated temperature, another method at room temperature was sought. Substitution of HCl for H_2SO_4 in the mixed reagent system was tried. With higher concentrations of HCl, the amount of precipitate decreased. However, the absorbance decreased significantly and this method was not acceptable. Some other unsuccessful attempts included addition of acetic acid and the chelating agent, disodium dihydrogen ethylenediamine tetraacetic acid.

The formation of the precipitate was eliminated after it was found that alcohol could act as a solvent in this case. The optimum amount of alcohol, which was found to be 1 ml per 20 ml sample, extended the range to about 2.00 mg/l PO_4 expressed at P in distilled and sea water. Further increase in alcohol content decreased the absorbance.

The addition of alcohol to the modified reagent system made it necessary to redetermine the spectral absorption characteristics of the molybdenum blue complex. Samples were run in distilled water and a 20,000 mg/l Cl^- solution using wave lengths ranging from 400 to 940 mμ. A concentration of 1.50 mg/l PO_4-P was used. The resulting data indicated the maximum absorbance peak occurred at about 880 mμ. Data from measurements at the wave lengths of 600 to 940 mμ are shown in Figure 1. Also shown are the results where alcohol was not added and the resulting precipitate was removed. This

Wavelength Characteristics of the
Modified-Alcohol Orthophosphate Procedure

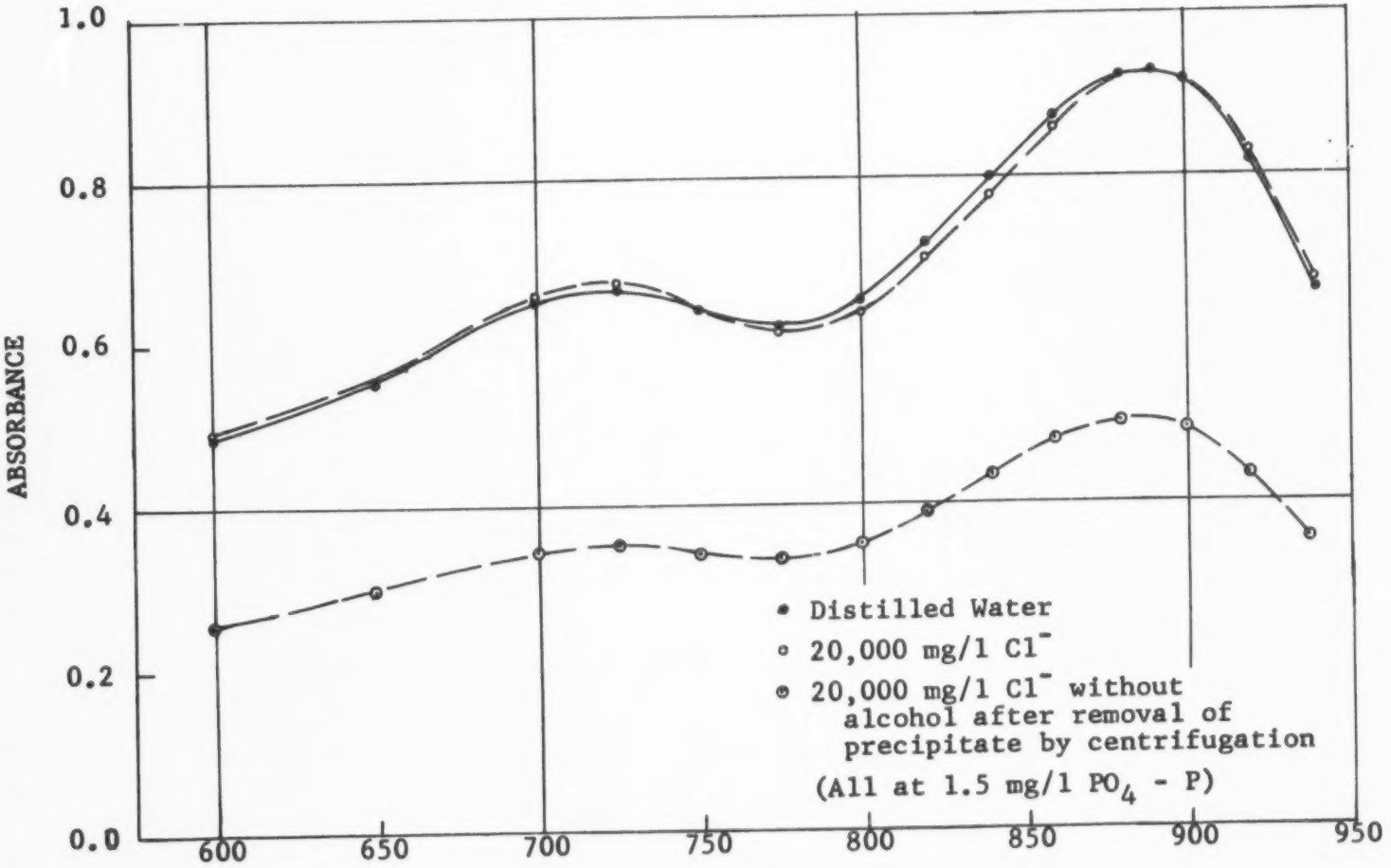

Figure 1

removal resulted in a 46% reduction in solution absorbance but did not change the spectral absorption characteristics of the complex.

The spectral data showed that the optimum wave length for both the modified-alcohol and the Murphy and Riley[5] methods was identical. The comparison of the two methods at the 880 mμ wave length, shown in Figure 2, illustrates the increased sensitivity and greater range of the modified-alcohol method.

Precision

Phosphate phosphorus was determined in twenty replicate samples containing 1.00 mg/1 of phosphate phosphorus (KH_2PO_4) in distilled water in order to compute the precision of the modified-alcohol method. A light path of one centimeter was used. The statistical data on the absorbance values are listed in Table 1.

Hydrolysis of Condensed Phosphates

Condensed phosphates such as those used in detergent formulations are easily hydrolyzed to orthophosphate and if hydrolysis occurs during analysis, the orthophosphate results will be high. Tests were made to determine the extent of hydrolysis of three condensed phosphates, sodium tripolyphosphate, tetrasodium pyrophosphate and sodium hexametaphosphate, in concentrations of 1.00 mg/1 as phosphorus in the presence of the reagent. Orthophosphate was used as a reference. The results in Table 2 show that the orthophosphate develops more than 99% of its color in four minutes. Using ten minutes as the time of reading, the pyrophosphate increased the result by 0.016 absorbance units while tripoly and hexametaphosphate increased the result by 0.011 and 0.012 absorbance units respectively. This is equivalent to 0.025, 0.017 and 0.019

Comparison of the Modified-Alcohol Method with
the Murphy and Riley Method (1962) for Orthophosphate Analysis

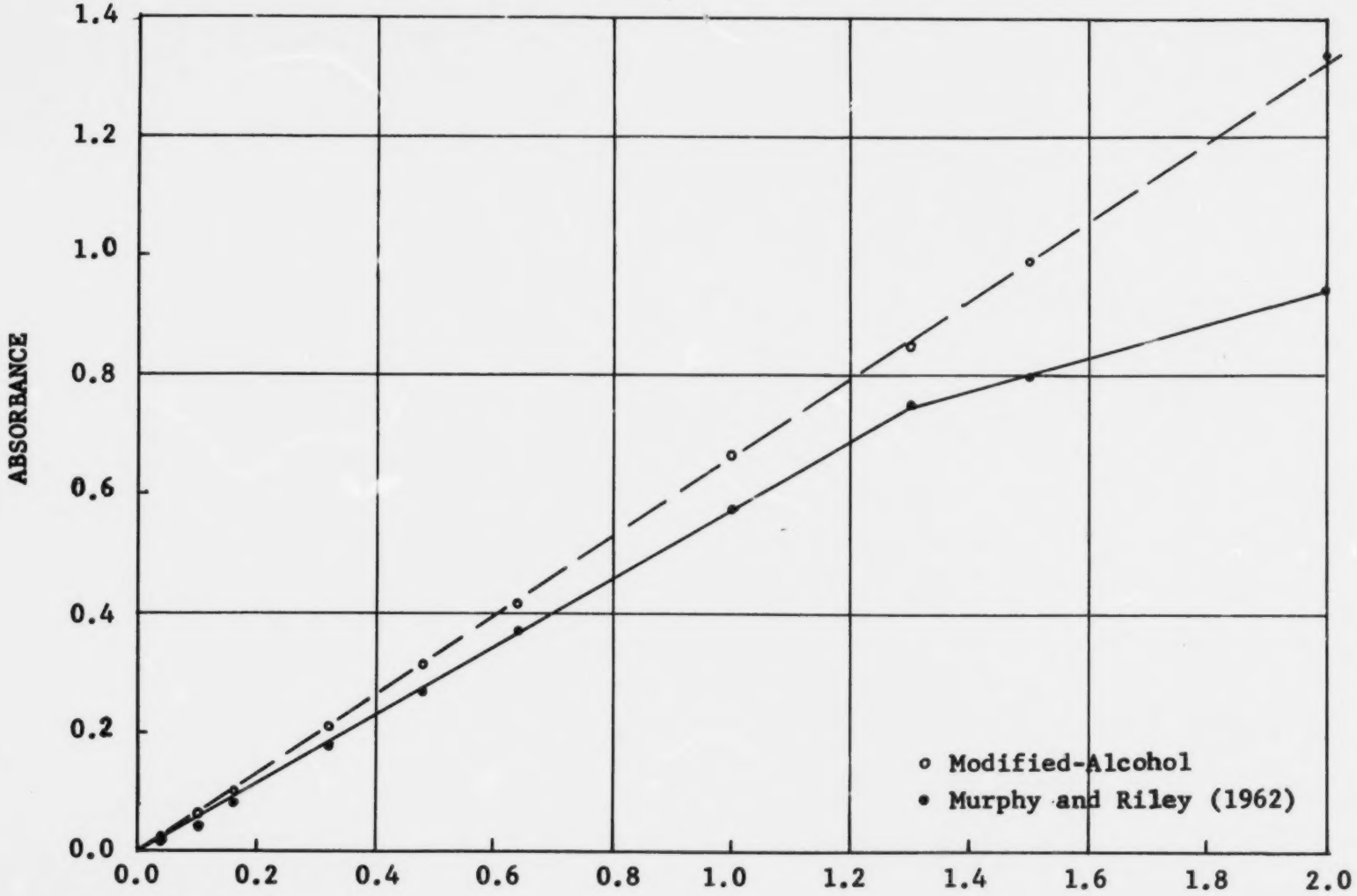

Figure 2

Table 1

Precision of Twenty Replicate Samples at 1.00 mg/l PO_4-P

Absorbance			
0.660	0.667	0.668	0.662
0.665	0.670	0.670	0.660
0.670	0.663	0.667	0.665
0.665	0.670	0.670	0.670
0.667	0.667	0.670	0.670

Mean ($\bar{x}$) = 0.667

Standard Deviation = $\pm$ 0.0034

95% Confidence Interval = ($\bar{x} \pm 1.96\ \sigma/\sqrt{n}$)

= $\bar{x} \pm 0.00152$

Table 2

Hydrolysis of Condensed Phosphates

	Absorbance Time After Addition of Reagent (Minutes)									
Phosphate*	3	4	5	7	10	15	30	45	60	120
Tripoly	0.009	0.010	.010	.010	.011	.012	.013	0.017	.020	.036
Pyro	0.009	0.010	.010	.013	.016	.021	.035	0.052	.067	.122
Hexameta	0.010	0.011	.011	.012	.012	.013	.014	0.017	.020	.035
Ortho	0.620	0.625	0.625	0.625	0.630	0.630	0.630	0.630	0.630	0.630

*All equivalent to a concentration of 1 mg/l PO_4-P.

mg/1 PO_4-P or 2.5, 1.7 and 1.9 per cent respectively based on 1.00 mg/1 P. The hydrolysis of a mixture of the more rapidly degraded pyrophosphate with orthophosphate, both at 1.00 mg/1 P concentration, was investigated to ascertain if a mixture had any effect on the hydrolytic rate of pyrophosphate. The results showed that the mixture had practically no effect on the rate of color development. The error was small, yielding 1.03 mg/1 instead of the correct 1.00 mg/1 P.

Salt Effect

According to Murphy and Riley,[8] most molybdenum blue methods for the determination of orthophosphate develop less intense colors with the same amount of phosphorus in sea water than they do in distilled water. The salt error is not a linear function of salinity. The difference is particularly large when the stannous chloride method is used and it may amount to 15 per cent. The computations of salt error based on the mean of ten sets of data for sea water and distilled water are shown in Table 3 for the modified-alcohol method. The values show that the salt error in phosphate phosphorus concentrations of 0.10 to 2.00 mg/1 is less than 2.5 per cent.

In certain tidal waters, the amount of fresh and sea water at one location will vary with the stage of the tide. Therefore, a calibration curve prepared with distilled water must be satisfactory for use over the entire range of salinity from distilled water to sea water. Calibration curves were prepared with distilled water, sea water, and the following mixtures of sea water and distilled water: 75, 50 and 25 per cent sea water. The data based on duplicate samples, as presented in Table 4, show that all five curves are practically identical, and that the distilled water calibration curve is satisfactory for waters with chloride concentrations up to 17.2 g/1.

Table 3

Salt Error

PO_4 as P mg/l	Absorbance		Salt Error %
	Distilled Water	Sea Water	
0.10	.063	.064	+ 1.6
0.48	.311	.318	+ 2.25
1.00	.661	.661	0.0
1.50	.991	.983	- 0.8
2.00	1.332	1.349	+ 1.27

Table 4

Salt Effect at Various Proportions of Sea Water and Distilled Water

Conc. of PO_4-P mg/l	Absorbance					
	Distilled Water	Sea Water	75% Sea Water	50% Sea Water	25% Sea Water	Mean of 10 Samples
0.04	.024	.023	.021	.023	.023	.0228
0.10	.064	.064	.062	.061	.062	.0626
0.48	.305	.308	.307	.305	.306	.3064
1.00	.635	.638	.635	.636	.638	.6362
1.50	.950	.954	.949	.954	.951	.9516
2.00	1.300	1.299	1.298	1.293	1.296	1.298

As a practical test of the method, a calibration curve was made using tidal salt water. A sample was collected from the Harlem River, a tidal salt water body connecting the Hudson River with the East River in New York City. The chloride content was 6.2 g/l. The color was high and the blank or the absorbance of the settled sample with reagents was 0.075, which is equivalent to about 0.12 mg/l phosphate expressed as P. A calibration curve was made by subtracting the blank absorbance value of 0.075 from the absorbances obtained by adding orthophosphate to the water. Essentially identical curves were obtained with distilled and Harlem River water. The mean values of six sets of data are shown in Table 5.

Interference

The effect of ions found in some waters which might interfere with this molybdenum blue method for phosphate determination was investigated. The ions included arsenic added as arsenate, cupric copper, silicon added as silicate, sulfide, nitrite, ferric iron and chromium added as dichromate. The ion concentrations were usually set at one and ten mg/l in solutions containing zero and 1 mg/l phosphate expressed as P. In the absence of phosphate, no significant increase in absorbance resulted from the addition of reagent to any of these ion solutions except arsenate. Arsenate reacts with molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.10 mg/l interfered with the test.

In the presence of 1 mg/l phosphate, silicate and sulfide had no effect but slight reductions in absorbance resulted from cupric copper and ferric iron. Chromate and nitrite decreased the absorbance about three per cent at 1 mg/l and 10-15 per cent at 10 mg/l.

Table 5

Absorbances of Orthophosphate Added to Harlem River and to Distilled Water

PO_4-P mg/l Added	Absorbance	
	Distilled Water	Harlem River
0.04	0.017	0.018
0.10	0.056	0.058
0.48	0.304	0.304
1.00	0.641	0.652
1.50	0.988	0.994
2.00	1.329	1.325

Manual Analysis of Sewage Samples

Early tests in fresh and sea water seemed to indicate that the procedure developed for water was not entirely satisfactory for sewage. Many changes were made in the reagents and in the best modification, the concentration of ascorbic acid was increased tenfold. The resulting calibration curve had a lower slope than the water reagent curve.

The advantage of having one method suitable for use in water and sewage containing various concentrations of salt is obvious. Further work was done on sewages and effluents using the procedure for water. Numerous samples of sewage and activated sludge effluent were collected from seven plants in the metropolitan New York area. Orthophosphate concentrations ranged from 2.75 to 13.9 mg/l. In every case, dilution was required to bring the spectrophotometric reading between 80 and 15 per cent transmission or absorbances of 0.1 and 0.82. For soluble phosphate, the sample should be centrifuged before testing. With dilution to bring the spectrophotometric reading within the proper range, turbidity and the usual sewage color does not interfere with the determination, and the absorbance obtained with the water procedure can be converted to mg/l using the calibration curve prepared with standards made up with distilled water.

The best way to evaluate the validity of the water procedure for use in sewage is to add known amounts of phosphate to sewage containing little or no phosphate and then attempt to recover the amount added. Since all samples tested contained too much phosphate for direct use, centrifuged samples were diluted to 10 per cent with distilled water. Three portions of each diluted sewage were taken. One was used as a blank and to the other two, enough phosphate was added to bring the added concentration to 0.5 and 1.0 mg/l P respectively. The reagents were added and the absorbances

measured. When the absorbance of the blank was subtracted from that of the sample containing the added phosphate, the difference represented the absorbance of 0.5 and 1.0 mg/1 P. Table 6 shows that the recovered amounts of phosphorus are very close to the amounts added. At the 1.0 mg/1 level, the individual recovered values were within 5 per cent of the added amount. The mean of the recovered amounts of phosphorus in the nine tests was 1.007 mg/1.

Sewage samples diluted with sea water were also tested and found to check closely the results obtained with the distilled water samples. Therefore, if sewage is discharged into fresh or salt water, the orthophosphate can be determined in the receiving water by comparing the absorbance of the sample with those obtained from the standards made up with distilled water. Some typical results with centrifuged raw sewage samples diluted with distilled water and with sea water are shown in Table 7. The average difference in absorbances between the distilled and the sea water values was 2.64 per cent based on the distilled water result.

The procedure for determining orthophosphate in sewage and effluent is shown in the Appendix.

The detailed procedure for determining orthophosphate in fresh and saline waters is presented in the Appendix.

A paper titled "Determination of Orthophosphate in Fresh and Saline Waters" has been accepted for publication in the Journal of the American Water Works Association.[9]

SUMMARY

1. A rapid, accurate and sensitive method for the determination of orthophosphate in fresh and saline waters, and sewage is presented.

2. The standard deviation from twenty replicate distilled water samples containing 1.00 mg/1 PO_4 as P was 0.51

Table 6

The Recovery of Measured Amount of Phosphate Added to Sewage

Sample			Absorbance						Absorbance		
1	2	3	4	5	6	7	8	9*	10	11	12*
No.	Type	% Dilution	Sample	0.5 mg/1 P	1.0 mg/1 P	Sample + 0.5 mg/1 P	Column 7-4	Recovered mg/1 P	Sample + 1.0 mg/1 P	Column 10-4	Recovered mg/1 P
1	Pri. Eff.	10	.325	.310	.640	.660	.335	.54	1.000	.675	1.05
2	Pri. Eff.	10	.560	.325	.660	.880	.320	.49	1.200	.640	.97
3	Pri. Eff.	10	.265	.335	.650	.610	.345	.52	.930	.665	1.02
4	Act. Sl. Eff.	10	.200	.320	.650	.535	.335	.52	.875	.675	1.02
5	Raw Sew.	10	.215	.322	.655	.540	.325	.51	.875	.660	.99
6	Raw Sew.	10	.275	.320	.650	.595	.320	.50	.925	.650	1.00
7	Raw Sew.	10	.185	.320	.650	.505	.320	.50	.850	.665	1.02
8	Pri. Eff.	10	.215	.320	.650	.540	.325	.51	.855	.640	.99
9	Act. Sl. Eff.	10	.210	.320	.650	.540	.330	.52	.870	.660	1.01

*Computed - based on absorbance for 0.5 and 1 mg/1 respectively.

Table 7

The Determination of Phosphate
in
Sewage and Effluents Diluted with Distilled and Sea Water

Centrifuged Sample	Distilled Water Dilution			Sea Water Dilution		
	% Sewage or Effluent	Absorbance	mg/l P	Absorbance	mg/l P	% Difference*
1. Raw Sew.	10	.205	3.0	.210	3.08	2.6
" "	20	.405	3.0	.425	1.12	5.0
2. " "	2	.187	14.0	.202	15.0	8.0
" "	5	.460	13.8	.472	14.1	2.6
" "	10	.930	13.9	.922	13.8	0.9
3. " "	10	.195	3.0	.193	3.0	1.0
" "	20	.415	3.15	.430	3.15	3.6
4. " "	10	.325	4.8	.332	4.8	2.2
" "	20	.660	4.8	.663	4.8	.5
5. " "	10	.275	4.15	.265	4.0	3.6
6. " "	10	.185	2.75	.183	2.75	1.9
7. Pri. Eff.	10	.215	3.20	.215	3.20	0
8. Act. Sl. Eff.	10	.210	3.15	.205	3.08	2.4

*Based on absorbance of distilled water sample.

per cent of the mean absorbance.

3. The salt error in sea water of concentrations between 0.10 and 2.00 mg/1 PO_4 as P is less than 2.5 per cent of the absorbance.

4. Calibration curves prepared with distilled water, sea water, and the following mixtures of distilled and sea water, 75, 50 and 25 per cent sea water, showed that the calibration curve prepared with distilled water is satisfactory in measuring orthophosphates in salt waters with concentrations of chloride up to 17.2 g/1.

5. The error from the hydrolysis of condensed phosphates to orthophosphate during analysis is negligible under the recommended conditions.

6. Preliminary tests indicate that the fresh water kjeldahl procedure for organic nitrogen is not satisfactory for use in salt water.

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APPENDIX

Method for Determining Orthophosphate in Fresh and Saline Waters, Sewage and Effluents

1. General Discussion

1.1. Principle: Ammonium molybdate and potassium antimonyl tartrate react in an acid medium with dilute solutions of orthophosphate to form a heteropoly acid, phosphomolybdic acid, which is reduced to the intensely colored molybdenum blue by ascorbic acid.

1.2. Interference: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.10 mg/l As^{V} interfere with the phosphate determination. Hexavalent chromium and nitrite interfere to give low results, about 3 per cent low at concentrations of 1.0 mg/l and 10 to 15 per cent at 10 mg/l of chromium and nitrite. Sulfide (Na_2S) and silicate does not interfere in concentrations of 1.0 and 10.0 mg/l.

1.3. Sampling and Storage: Phosphate samples and solutions must not be collected or stored in plastic containers because phosphates are adsorbed by plastic.

2. Apparatus

2.1. Acid-washed Glassware: All glassware used in the determination should be filled with concentrated sulfuric acid, allowed to stand overnight and then rinsed thoroughly. Preferably this glassware should be used only for the determination of phosphate and

after use, it should be washed and kept filled with water until needed again. If this is done, the treatment with sulfuric acid is only required occasionally. Commercial detergents containing phosphates should be avoided.

2.2. Colorimetric Equipment:

a. Spectrophotometer with infrared phototube for use at 880 mμ providing a light path of 0.5 cm or longer.

3. Reagents

3.1. Sulfuric Acid Solution, 5N. Dilute 70 ml conc H_2SO_4 with distilled water to 500 ml.

3.2. Potassium antimonyl tartrate. Dissolve 4.3888 g $K(SbO)C_4H_4O_6 \cdot 1/2\ H_2O$ in 200 ml distilled water. Store in dark bottle at 4°C.

3.3. Ammonium Molybdate Solution. Dissolve 20 g $(NH_4)_6\ Mo_7\ O_{24} \cdot 4\ H_2O$ in 500 ml distilled water. Store in a plastic bottle at 4°C.

3.4. Ascorbic Acid, 0.1M. Dissolve 1.76 g ascorbic acid in 100 ml distilled water. Solution is stable for about a week if stored at 4°C.

3.5. Combined Reagent. Mix the above reagents (3.1, 3.2, 3.3 and 3.4) in the following proportions for 100 ml of the combined reagent: 50 ml of 5N sulfuric acid; 5 ml of potassium antimonyl tartrate solution; 15 ml of ammonium molybdate solution; and 30 ml of ascorbic acid solution. All reagents must reach room temperature before they are mixed and it is recommended that they be mixed in the order given. If turbidity forms in the combined reagent after the addition of potassium antimonyl tartrate or ammonium molybdate, shake the combined reagent and let it stand for a few minutes until the turbidity

disappears before proceeding. The reagent is stable for at least one week if stored at 4°C.

3.6. Alcohol, ethyl (95%) or isopropyl.

3.7. Stock Phosphate Solution: Dissolve in distilled water 0.1757 g potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml: 1 ml = .040 mg P.

3.8. Working Phosphate Standard: Dilute 50.0 ml stock phosphate solution to 1000 ml with distilled water. This solution contains 0.002 mg P per 1.00 ml.

4. Procedure

4.1. If necessary, filter the sample as directed in Standard Methods (11th ed.), p. 201, Method A, Sec. 4.1, or centrifuge the sample.

In the case of sewage, the sample usually requires dilution to bring the absorbance of the treated sample within the proper range for reading. Dilution after clarification helps to minimize interference from turbidity or color.

4.2. Pipet 20.0 ml of clear sample into a clean dry test tube or 125 ml erlenmeyer flask. Add 1 ml of ethyl or isopropyl alcohol. Mix thoroughly. Since curves prepared with ethyl alcohol are slightly different from those prepared with isopropyl alcohol, the same alcohol must be used in treating samples and standards. Add 1 ml of combined reagent. Mix thoroughly and allow to stand 10 minutes for color development before reading in a spectrophotometer at a wave length of 880 mμ. Natural color of water generally does not interfere at the high wave length used. In the case of highly colored or turbid waters, a blank may be prepared by adding all of the reagents except ascorbic acid and potassium antimonyl

tartrate to the sample. The absorbance of the blank should be subtracted from the absorbance of each of the unknown samples. The calibration curve uses a distilled water blank with the complete reagent. Light path lengths for readings between absorbances of 0.1 and 0.82 or transmissions of 80 and 15% are suitable for the following phosphate ranges computed as P.

Approx. P Range mg/l	Light Path cm
0.30 - 2.0	0.5
0.15 - 1.30	1.0
0.03 - 0.25	5.0

4.3. Obtain the concentration or weight of orthophosphate computed as P in the sample by referring the reading of absorbance to a standard curve. This curve is obtained by plotting on arithmetic graph paper the absorbance against the concentration of orthophosphate as P for a suitable number of phosphate standards. The plot should be a straight line passing through the origin. At least one standard should be tested with each set of samples or once each day that tests are made.

5. Calculation

$$\text{mg/l P} = \frac{\text{mg P} \times 1000}{\text{ml sample}}$$

If phosphate as PO_4 is desired, multiply the result as P by 3.06.

6. Precision: The standard deviation of the absorbances based on twenty replicate samples containing 1.00 mg/l phosphate phosphorus was $\pm$ 0.0034 which is .51 per cent of the mean absorbance. This is equivalent to 1.00 $\pm$ .005 mg/l.